An insight into the possible protective effect of pyrrolidine dithiocarbamate against lipopolysaccharide-induced oxidative stress and acute hepatic injury in rats

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Abstract Lipopolysaccharide (LPS) is a major cell wall molecule of Gram-negative bacteria known to stimulate the synthesis and secretion of several toxic metabolites, such as reactive oxygen species. In this study, the effect of pyrrolidine dithiocarbamate (PDTC), an antioxidant with nuclear factor-κB inhibitor activity, was evaluated in LPS-induced oxidative stress and acute hepatic injury in rats. Animals were pretreated for 3 consecutive days with PDTC (200 mg/kg/day, i.p.) or saline and animals were then challenged with LPS (6 mg/kg, i.p.) or saline. Six hours after LPS injection, animals were decapitated and blood and liver samples were collected to assess the chosen biochemical parameters. Saline-pretreated animals challenged with LPS revealed extensive liver damage, as evidenced by increases in serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gamma glutamyl transferase (γ-GT). Also, LPS treatment resulted in significant increases in serum lactate dehydrogenase (LDH), tumor necrosis factor-alpha (TNF-α) and nitrite levels. Furthermore, LPS challenge caused oxidative stress as indicated by an increase in hepatic lipid peroxidation measured as thio-barbituric acid reactive substances (TBARS) and a decrease in hepatic reduced glutathione concentration (GSH) as well as decreased activities of superoxide dismutase (SOD) and catalase in hepatic tissues. The administration of PDTC prior to LPS challenge resulted in improved liver functions as evidenced by the decline in serum AST, ALT, γ-GT levels and reduction in serum LDH, TNF-α and nitrite levels. Moreover, PDTC reduced the chosen lipid peroxidation marker, TBARS and increased GSH concentration, and SOD and catalase

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1. Introduction

Lipopolysaccharides (LPS) are glycolipids found in abundance on the outer membrane of all Gram-negative bacteria and have the ability to incite a vigorous inflammatory response (Su, 2002). LPS have been recognized for many years as a key risk factor in the development of septic shock syndrome (Jaeschke, 2000; Jaeschke et al., 2002). Challenging of experimental animals with LPS causes a wide spectrum of pathophysiological changes which parallel those observed in patients with Gram-negative bacterial infections (Glauceri et al., 1991).

Gram-negative bacteria normally colonize the colon, so the body has developed strong defensive mechanisms that tightly regulate the entry and processing of LPS. The liver plays a central role in this process (Su, 2002). The vast majority of LPS that enters to the host in normal and pathological states does so through the gastrointestinal tract (GIT). Strategically and uniquely located at the gateway of the portal blood flow draining the GIT, the liver is the final barrier to prevent gastrointestinal bacteria and bacterial products such as LPS, from entering the systemic blood stream (Su, 2002). Systemic administration of LPS generally leads to its rapid accumulation in hepatic Kupffer cells, which are the major cell type for the detoxification of the LPS endotoxin (Hewett and Roth, 1993). Hepatic Kupffer cells comprise approximately 70% of the total macrophage population of the body. Under normal conditions, Kupffer cells can efficiently clear non-pathogenic amounts of circulating LPS (Hewett and Roth, 1993). After challenging of experimental animals with LPS, it is taken up rapidly by the liver within 5 min in rabbits (Mathison and Ulevitch, 1979). In rats, hepatectomy (90%) resulted within 2 h in bacteremia due to bacterial translocation, although the phagocytic function of the lung and the spleen tissue cells increased immediately (Wang et al., 1993). Therefore, the liver appears to be a potent scavenger of circulating bacteria and their products. Mild hepatic dysfunction might promote systemic endotoxin spillover and the subsequent inflammatory process, suggesting the importance of adequate liver function to patient survival (Chaudry et al., 1986). Despite the ability of the liver to detoxify LPS, marked morphological and biochemical alterations can occur in hepatic tissues exposed to vast amounts of LPS (Freudenberg and Galanos, 1992). Patients and experimental animals died from endotoxemia displayed alterations in Kupffer cells, formation of fibrin thrombi, neutrophil infiltration in liver sinusoids, and zonal hepatic necrosis (Hewett and Roth, 1993; Freudenberg and Galanos, 1992).

Endotoxemia occurs frequently in cases of liver failure and is thought to play a role in the pathogenesis of liver disease (Nakao et al., 1994). LPS interaction with the hepatic phagocytes namely, Kupffer cells is usually associated with their activation and subsequently the release of a plethora of mediators (Decker, 1990) including reactive oxygen metabolites (ROM), products of lipid metabolism (Jaeschke, 2000), and nitric oxide (NO) Zhang et al., 2000 as well as proinflammatory mediators, such as tumor necrosis factor-α (TNF-α) Leist et al., 1995 which are responsible for the recruitment and activation of neutrophils and other inflammatory cells known to augment tissue injuries (Erzurum et al., 1992). All these mediators have raised a considerable interest in recent years for their possible roles in explaining the mechanisms of LPS-induced acute tissue injuries.

Over the past few decades and, despite advances in antimicrobial therapy and critical care medicine, sepsis-related systemic inflammatory syndrome and multiple organ dysfunction continue to be the most common cause of morbidity and mortality in intensive care units, reaching a mortality rate of nearly 50%. Therefore, it is imperative to develop new drugs or strategies that can arrest the many indolent and deadly consequences of endotoxemia.

The dithiocarbamates are a class of antioxidants reported to be potent inhibitors of nuclear factor kappa B (NF-κB) both in vivo and in vitro (Shreck et al., 1992; Cuzzocrea et al., 2002). The most potent member of this class is pyrrolidine dithiocarbamate (PDTC) that has been identified as a potent antioxidant and has been used to counteract the toxic effects of free radicals and to interfere with the generation of proinflammatory cytokines (Liu and Malik, 1999). PDTC has been advocated for the treatment of acquired immune deficiency syndrome (AIDS) in HIV-infected patients and neurodegenerative diseases (Sunderman, 1991). Although PDTC has been shown to have both anti-inflammatory and antioxidant activities (Shreck et al., 1992; Tsuchihashi et al., 2003) yet its effects on oxidative stress induced by endotoxin intoxication in the liver has not been fully elucidated. The current investigation aimed to study the effect of PDTC on oxidative stress and acute hepatic injury induced by LPS administration in rats and to characterize the possible mechanisms by which PDTC might attenuate endotoxin-induced hepatic injury. In the present study, hepatic dysfunction was assessed by measuring serum levels of alanine aminotransferase (ALT, a specific marker for hepatic parenchymal injury), aspartate aminotransferase (AST, a non-specific marker for hepatic parenchymal injury), gamma glutamyl transferase (γ-GT, a marker for cholestasis) and lactate dehydrogenase (LDH, an indicator of cytotoxicity). The present study also investigated the effects of PDTC on serum nitrite and tumor necrosis factor-α (TNF-α) levels as well as oxidative stress markers in an effort to gain better insight into the mechanisms of the protective effects of PDTC in LPS-challenged rats.

2. Materials and methods

2.1. Chemicals

Lipopolysaccharide ([Escherichia coli LPS 0127:B8]) lyophilized powder chromatographically purified by gel filtration (protein content <1%), pyrrolidine dithiocarbamate, N-(1-naphthyl) ethylenediamine dihydrochloride, sulfanilamide, 5,5-dithi-2-
nitrobenzoic acid, thiobarbituric acid and 1,1,3,3-tetramethoxypropane in addition to all other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Kits used to measure ALT, AST, γ-GT and LDH were purchased from Spinreact Inc., (France). Superoxide dismutase activity was measured using Ransod kit (Randox Laboratories Ltd., Antrim, UK). TNF-α kit was obtained from R&D system (Minneapolis, MN, USA).

2.2. Animals

Adult male Sprague–Dawley rats weighing 200–220 g were obtained from the animal house, College of Pharmacy, King Saud University. All animals were housed in stainless-steel cages with free access to pelleted Purina laboratory chow and tap water. The rats were kept at room temperature (22–24 °C) and 55 ± 5% relative humidity with a regular 12-h light and 12-h dark cycle. Animals used in this study were treated and cared for in accordance with the strict guiding principles of the National Institution of Health for experimental care and use of animals. The experimental design and procedures were approved by the Institutional Ethical Committee for Animal Care and Use at the King Saud University, Riyadh, Kingdom of Saudi Arabia.

2.3. Experimental design

Animals were randomly allocated into four groups, 8 animals each. Group I (control group) treated with an equivalent volume of saline alone. Group II (PDTC-treated group) received PDTC (200 mg/kg/day, i.p.) for 3 consecutive days. Group III (LPS-treated group) challenged with a single dose of LPS (6 mg/kg, i.p.) Collin et al., 2004. Group IV (PDTC and LPS-treated group) pretreated with 3 doses of PDTC (200 mg/kg/day, i.p.) 48, 24 and 1 h before being challenged with LPS (6 mg/kg, i.p.). PDTC and LPS were dissolved in saline shortly before use. The dose and duration of PDTC treatment was chosen based on previous studies (El Eter et al., 2005; Hagar et al., 2007) as well as preliminary experiments.

2.4. Collection of samples

Six hours after LPS injection, animals were decapitated; blood samples were collected and sera were separated by centrifugation at 3000g for 10 min and then stored at −70 °C. Collected samples were used within 48 h for the determination of the chosen biochemical parameters. Livers were isolated and treated as described below.

2.5. Hepatic tissue preparation

Livers were excised from animals immediately after decapitation and rinsed with ice-cold saline to remove excess blood. All subsequent steps were carried out at 4 °C. Following rinsing, livers were finely minced. Approximately 1 g of hepatic tissues was homogenized with a Brinkmann Polytron in a sufficient volume of ice-cold 50 mM potassium phosphate buffer, (pH 7) to produce a 10% (w/v) homogenate which was centrifuged at 3000g for 10 min. The supernatants were frozen in liquid nitrogen and kept at −80 °C for the subsequent assessment of lipid peroxidation marker and antioxidant enzymes.

2.6. Measurement of serum liver function enzymes

Hepatic dysfunction was assessed by measuring the rise in serum levels of ALT, AST and γ-GT using commercially available kits. The results were expressed as U/L.

2.7. Measurement of serum LDH

Serum lactate dehydrogenase (LDH) level was assessed using commercially available kit according to the manufacturer’s direction. The results were expressed as U/L.

2.8. Measurement of serum nitrite

Serum nitrite level was measured following the Griess reaction (Green et al., 1981). Serum samples were diluted and deproteinized by adding 1/20th volume of 30% zinc sulphate solution. After centrifugation of the samples at 3000g for 15 min 100 μl of supernatants were incubated with 100 μl of Griess reagent (0.1% N-(1-naphthyl) ethylenediamine dihydrochloride; 1% sulfanilamide in 5% phosphoric acid) at room temperature for 20 min. The absorbance was measured spectrophotometrically (Lambda 5, Perkin–Elmer, Promona, USA) at 550 nm. The concentration of nitrite was calculated using sodium nitrite as a standard and expressed as μmol/L.

2.9. Estimation of serum TNF-α

Serum TNF-α level was measured by an enzyme-linked immunosorbent assay (ELISA) kit (rat TNF-α ELISA) kit was obtained from R&D system (sensitivity: < 10 pg ml⁻¹), according to the instructions of the manufacturer. Briefly, after thawing 50 μl of serum samples were diluted 1:2 with the provided diluents to stay within the linear range of the assay. The samples were then incubated in micro-wells coated with mouse anti-rat TNF-α for 2 h at room temperature. After repeating the aspiration and washing for four times, 100 μl of the biotinylated goat anti-rat TNF-α was added to each well and incubated for 2 h. After washing, 100 μl of streptavidin conjugated to horseradish peroxidase was added to the wells and incubated for 20 min. Following a wash to remove unbound antibody-enzyme reagent, 100 μl of substrate solution was added. The color intensity of the enzymatic indicator reaction was measured photometrically at 450 nm in an ELISA plate reader (ELx 808 Ultra Microplate Reader BIO-TEK Instruments Inc., USA) and results were expressed as pg/ml.

2.10. Determination of hepatic lipid peroxidation

Lipid peroxidation, as an indicator of oxidative stress, was estimated by measuring thiobarbituric acid reactive substance (TBARS) that sometimes referred to as malondialdehyde (MDA) in hepatic homogenates as previously described (Buege and Aust, 1978). Briefly, 1 ml of 10% liver homogenate was reacted with 2 ml of fresh solution of thiobarbituric acid (TBA) reagent containing 0.375% TBA, 15% tetrachloroacetic acid (TCA), and 0.25 N HCl. The mixture was heated at 95 °C for 15 min, and then cooled to room temperature using tap water and centrifuged at 3000g for 10 min. Absorbance of the pink supernatant was determined spectrophotometrically at 532 nm. The amount of MDA was expressed as nmol/g wet hepatic tissue using 1,1,3,3-tetramethoxypropane as a standard.
2.11. Measurement of hepatic superoxide dismutase

Hepatic superoxide dismutase (SOD) activity was measured according to the method described by Sun et al. (1988). The principle of the method is based on the inhibition of nitroblue tetrazolium (NBT) reduction by the xanthine–xanthine oxidase system as a superoxide generator. One unit of SOD was defined as the enzyme amount causing 50% inhibition in the NBT reduction rate. The absorbance was measured at 505 nm and SOD activity was expressed as U/mg wet hepatic tissue.

2.12. Measurement of hepatic catalase

Hepatic catalase activity was determined by a colorimetric method based on the decomposition of hydrogen peroxide (H₂O₂) (at 20 °C, pH 7.0), by catalase, which can be followed directly by the decrease in the absorbance at 240 nm (Higgins and Buchner, 1978). Sample (50 μl) was placed in 900 μl phosphate buffer (50 mM, pH 7.0) and subsequently the reaction was started by the addition of 50 μl H₂O₂ (30 mM, in phosphate buffer) to the sample cuvette. The decrease in absorbance at 240 nm was followed for every min for about 2 min. The specific activity of catalase was expressed as the number of μmol H₂O₂ decomposed/min/mg wet hepatic tissue.

2.13. Determination of hepatic reduced glutathione

Reduced glutathione (GSH) concentration in hepatic homogenate was determined by the method of Ellman (1959). Proteins in the samples of 0.5 ml liver homogenate (10%) were precipitated by the addition of 0.5 ml of 15% TCA solution. After 40 min, the protein precipitate was separated from the remaining solution by centrifugation at 1000 g at 4 °C for 5 min. Supernatant (200 μl) was combined with 1.7 ml of phosphate buffer (50 mM, pH 8.0) and 100 μl of Ellman’s reagent (5,5-dithio-2-nitrobenzoic acid) in phosphate buffer and the absorbance was measured spectrophotometrically at 412 nm. The GSH concentration was determined using a standard curve constructed with different concentrations of an authentic sample. The results were expressed as nmol/g wet tissue weight.

2.14. Statistical analysis

The degree in variability of results was expressed as means ± SEM. Data were evaluated by one-way analysis of variance (ANOVA) followed by Tukey–Kramer multiple comparisons test. The level of significance was accepted at P < 0.05.

3. Results

3.1. Effects of PDTC on LPS-induced liver injury

Injection of LPS in a dose level of 6 mg/kg, i.p. resulted in a considerable hepatic injury as assessed by elevations of serum ALT and AST and γ-GT levels (Fig. 1A–C). These markers were substantially elevated by about sevenfold, ninefold and threefold, respectively, when compared with that of salinetreated animals. Three consecutive days prior administration of PDTC (200 mg/kg/day, i.p.) significantly decreased the LPS-induced elevations in serum ALT, AST and γ-GT levels.
by about 52.13%, 73.1% and 55.57%, respectively (Fig. 1A–C).

3.2. Effect of PDTC on serum LDH level in LPS-treated rats

Serum LDH level was significantly greater after LPS administration than saline-treated control group by about 202.2%, suggesting some sort of membrane instability induced by endotoxin (Fig. 2). Treatment of rats with PDTC (200 mg/kg/day, i.p.) for 3 consecutive days significantly decreased the elevation in serum LDH level by about 31.31% as compared to animals treated with LPS alone (Fig. 2).

3.3. Effect of PDTC on serum TNF-α level in LPS-treated rats

Injection of LPS at a dose level of 6 mg/kg, i.p. resulted in significant increase in serum TNF-α level compared to saline-treated control animals (Fig. 3). Prior administration of PDTC (200 mg/kg/day, i.p.) for 3 consecutive days significantly reduced the elevation in serum TNF-α level by about 42.1% as compared to LPS-treated group (Fig. 3).

3.4. Effect of PDTC on serum nitrite level in LPS-treated rats

Induction of endotoxemia by LPS resulted in a significant elevation in serum nitrite level by about 11.1-fold compared to the control group value (Fig. 4). Treatment with PDTC (200 mg/kg/day, i.p.) for 3 consecutive days prior to induction of endotoxemia significantly reduced the serum nitrite level to 23.44% in comparison with the corresponding value in LPS-treated rats alone (Fig. 4).

3.5. Effect of PDTC on hepatic MDA concentration in LPS-treated rats

As illustrated in Table 1, rats injected with a single dose of LPS (6 mg/kg, i.p.) caused an increase in the formation of lipid peroxides, measured as MDA in hepatic tissues, by about 288%. Pre-treatments with PDTC (200 mg/kg/day, i.p.) for 3 consecutive days significantly attenuated LPS-evoked increase in MDA concentration reaching to a value of 52.7% compared to that in group treated with LPS alone (Table 1).

3.6. Effect of PDTC on hepatic GSH concentration in LPS-treated rats

The effect of PDTC on GSH concentration in LPS-treated animals is presented in Table 1. Results showed that LPS decreased GSH content by about 40.8% in the liver as compared to saline-treated control rats. Prior treatment with PDTC (200 mg/kg/day, i.p.) for 3 consecutive days was able to significantly increase the hepatic concentration of GSH up to
168.1% as compared to the corresponding value of LPS-treated group.

3.7. Effect of PDTC on hepatic SOD activity in LPS-treated rats

Hepatic SOD activity was significantly decreased in the samples of LPS-treated rats by about 36.2% in comparison with the control group (Table 1). Administration of PDTC (200 mg/kg/day, i.p.) for 3 consecutive days before LPS injection protected from LPS-induced decline in hepatic SOD activity reaching a value of 145.5% as compared to the corresponding value of LPS-treated group.

3.8. Effect of PDTC on hepatic catalase activity in LPS-treated rats

Hepatic catalase activity was significantly lowered in the LPS-treated group reaching about 48.2% when compared with the value of saline-treated control group (Table 1). However, catalase activity was increased in LPS group pretreated with PDTC (200 mg/kg/day, i.p.) for 3 consecutive days up to 170.7% compared with the value of LPS-treated group alone.

3.9. Effect of PDTC on treatment on the measured biochemical parameters

No significant changes were observed in the measured parameters in group treated with PDTC alone.

4. Discussion

Hepatic dysfunction after sepsis is a frequent event that is characterized by loss of synthetic function, hepatocellular necrosis, and release of inflammatory mediators such as TNF-α, interleukin-1β, interleukin-6, and NO (Zhang et al., 2000). The present study demonstrates that LPS-induced endotoxemia resulted in impairment in liver functions that was manifested by increased serum ALT, AST and γ-GT levels. Moreover, serum levels of LDH, nitrite and TNF-α were elevated by LPS administration. Endotoxemia also induced oxidative stress that was revealed by increased hepatic TBARS, a marker of lipid peroxidation, decline in hepatic GSH concentration and SOD and catalase activities. Prior administration with PDTC, an antioxidant with NF-κB inhibitor activity alleviated LPS-induced impairment in liver functions and reduced serum LDH, nitrite and TNF-α levels. Moreover, PDTC was able to provide protection against LPS-induced oxidative stress.

Proinflammatory cytokines play an important role as mediators in the pathophysiology of various diseases including endotoxin-induced shock (De la Fuente and Victor, 2001). In the present study, LPS-induced an increase in serum TNF-α level and this was dramatically attenuated by PDTC treatment. TNF-α is a potent inflammatory cytokine produced by the monocyte-macrophage lineage, including hepatic Kupffer cells (Enomoto et al., 2003). It has been reported that with LPS model, TNF-α is the central proximal mediator, which controls all subsequent events (Jaeschke, 2000; Enomoto et al., 2003). TNF-α can induce the production of other cytokines, adhesion molecules, arachidonic acid metabolites. Liver is the major source for the increase in serum TNF-α in endotoxin-induced shock in animals (Kumins et al., 1996). Inhibition of TNF-α synthesis or activity attenuates liver injury caused by LPS (Jaeschke et al., 2002) indicating that TNF-α is a critical factor in the sepsis-related liver toxicity. The current study revealed that treatment with PDTC reduced serum TNF-α, suggesting antiinflammatory action which would partially contribute to liver protection.

PDTC bears a numbers of beneficial properties including antioxidant activity, antiinflammatory and immunomodulatory effects (Cuzzocrea et al., 2002), all of which could account for the observed protection afforded by PDTC in this study. Moreover, PDTC can traverse the cell membrane and has prolonged stability in solution at physiological pH; effects that may account for its protective actions (Topping and Jones, 1988). In addition, PDTC can inhibit the activation of the nuclear transcription factor NF-κB produced by LPS which could result in a decrease of TNF-α synthesis (Rahman et al., 1998). Previous studies have indicated a major role for the NF-κB in the induction of TNF-α gene by endotoxin (Rahman et al., 1998; Collart et al., 1990). PDTC protected against different models of inflammation including angiotensin II-induced inflammation (Muller et al., 2000), carrageenan-induced pleurisy (Cuzzocrea et al., 2002), experimental model of ulcerative colitis (Hagar et al., 2007) and inflammatory conditions induced by ischemia/reperfusion to organs as kidney (Chatterjee et al., 2003), stomach (El Eter et al., 2005) and intestine (Tian et al., 2006).

Besides TNF-α, it has been suggested that ROM as singlet oxygen, NO, hydrogen peroxide and radicals such as superoxide anion and hydroxyl radicals are involved in the pathogenesis of endotoxic shock (Morgan et al., 1988). ROM are important cytotoxic and signaling mediators in the pathophysiology of

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<th>TBARS (nmol/g)</th>
<th>GSH (nmol/g)</th>
<th>SOD (U/mg)</th>
<th>Catalase (μmol/min/mg)</th>
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</thead>
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<tr>
<td>Control</td>
<td>25.4 ± 2.11</td>
<td>3125 ± 113</td>
<td>8.12 ± 0.27</td>
<td>4.75 ± 0.12</td>
</tr>
<tr>
<td>PDTC</td>
<td>27.23 ± 3.10</td>
<td>3043 ± 110</td>
<td>8.46 ± 0.19</td>
<td>4.86 ± 0.17</td>
</tr>
<tr>
<td>LPS</td>
<td>72.43 ± 5.22*</td>
<td>1850 ± 116*</td>
<td>5.18 ± 0.19*</td>
<td>2.29 ± 0.13*</td>
</tr>
<tr>
<td>LPS + PDTC</td>
<td>38.31 ± 4.13*</td>
<td>3110 ± 115#</td>
<td>7.54 ± 0.32#</td>
<td>3.91 ± 0.11#</td>
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Values are expressed as means ± SEM, n = 8.

* Significant difference from control group (P < 0.001).

# Significant difference from LPS-treated group (P < 0.001).
inflammatory liver diseases (Jaeschke, 2000) and play an important role in the onset of hepatic damage during endotoxemia or sepsis (Spolars, 1998). ROM can cause cellular injury via several mechanisms including the peroxidation of membrane lipids and the oxidative damage of proteins and DNA (Halliwell and Gutteridge, 1990).

Lipid peroxidation is produced by the attack of ROM upon the cell membrane resulting in membrane fluidity and permeability, which eventually leads to oxidative destruction of cellular membranes and cell lysis (Halliwell and Gutteridge, 1990). In this study, the results clearly showed that LPS could induce production of ROM measured indirectly by elevated hepatic TBARS. It is possible that the damage inflicted by lipid peroxidation upon lysosomal membranes in animals exposed to LPS enhanced the leakage of hydrolytic enzymes, and thus can severely injure the cells or tissues. Moreover, products of lipid peroxidation are potent chemotactic factors for neutrophils and can modulate their reactive oxygen formation (Curzio et al., 1986). They may also be involved in the generation of chemokines (Jayatilke and Shaw, 1998). These finding indicate that lipid peroxidation products are the determining factor for the continuation of neutrophils recruitment and aggravation of the injury (Wang et al., 1996). In this study, PDTC protected against LPS-induced lipid peroxidation as indicated by reduction in hepatic TBARS and thereby supported antioxidant activity of PDTC. Indeed inhibition of lipid peroxidation improves survival rate of endotoxemic rats (Kumimoto et al., 1987). PDTC functions as an antioxidant due to two structural features: direct scavenging of ROM by the dithiocarbonyl group, and chelating activity for heavy metal ions that may catalyze formation of ROM. In addition, PDTC protects against NF-κB-mediated pathological effects induced by various stimuli including LPS and cytokines (Liu and Malik, 1999; Rahman et al., 1998). ROM has been reported to be a crucial event in the activation of NF-κB (Shreck et al., 1992). Therefore, it has been proposed that antioxidants, by quenching ROM, may in turn block NF-κB activation and finally inhibit TNF-α production.

The cells are normally equipped with protective cell defense mechanisms, which include SOD, catalase, and GSH (Iszard et al., 1995). The balance between prooxidant production and antioxidant defense is pivotal for a correct cell function, whereas a disturbance in this balance in favour of the oxidants represents an oxidative stress. Several reports indicate that, tissue injury induced by various stimuli, including sepsis is coupled with GSH depletion (Hsu et al., 2004). The present study also demonstrated that sepsis yields in depletion of the hepatic GSH stores. With inflammation, GSH level in the liver decreases (Paradkar et al., 2004). LPS could have caused consumption of GSH to protect against ROM production or it may cause efflux of intracellular GSH, as suggested by others (Jaeschke, 1992). It is well known that GSH, the main intracellular nonprotein sulfhydryl, plays an important role in the maintenance of cellular proteins and lipids in their functional state, and provides major protection in oxidative injury by participating in the cellular defense systems against oxidative damage (Jefferies et al., 2003).

In addition to GSH, SOD and catalase constitute the defense antioxidant system against ROM. In the current investigation, LPS-induced a decline in hepatic SOD and catalase activities. These results are in accordance with previous studies that have been suggested that ROM production mainly superoxide anion and hydroxyl radical, may be a major cause of LPS-induced oxidative stress (Hsu et al., 2004). LPS may activate neutrophils to produce a number of short-lived ROM including superoxide anion, hydrogen peroxide and hydroxyl radical (Bautista et al., 1990; Beckman et al., 1990). PDTC administration prevented the depletion of SOD and catalase in hepatic tissues, thus it appears that the protective effect of PDTC may involve the maintenance of antioxidant capacity in protecting tissues against LPS-induced oxidant stress.

In the present study, LPS administration in rats resulted in remarkable increase in serum nitrite level, as a measure of NO production, which was attenuated by PDTC-pretreatment. Under normal conditions, it is well established that NO levels are low; however, the administration of LPS may cause the induction of NO synthase (iNOS), resulting in the production of large quantities of NO in many organs including the liver (Zhang et al., 2000). In the liver, LPS-activated Kupffer cells, endothelial cells, and hepatocytes are known to be a source of iNOS-derived NO (Duval et al., 1996; Rockey and Chung, 1996). In addition, inflammatory cells such as phagocytic leukocytes express iNOS when appropriately stimulated by cytokines (e.g. TNF-α and IL-1β) or by LPS (Rachmilewitz et al., 1995). Sustained production of NO after LPS challenge may cause hepatocellular injury either directly or indirectly. NO itself, or through one of its metabolites, can promote oxidative stress and thus provide an environment favouring the generation of more reactive species leading to hepatocellular injury. NO may participate in the development of oxidant stress in a number of ways. The loss of a single electron generates the highly reactive nitrosyl cation (NO1) (Stamler, 1984). In addition, the reaction of NO with superoxide radical generates peroxinitrite ONOO2 (Cuzzocrea et al., 2006). Both NO1 and ONOO2 are potent oxidants that can react with lipids, proteins, DNA, and GSH (Stamler, 1984). Generation of these reactive species could also explain the increased lipid peroxidation and the loss of GSH and the appearance of markers of oxidant stress. In the current study, PDTC reduced serum nitrite level and protected against LPS-induced oxidative hepatic injury. These results confirm the role of NO in LPS-induced liver injury. Inhibition of NO synthesis greatly reduced oxidant stress in the liver after LPS administration (Zhang et al., 2000). Although the mechanisms by which PDTC attenuated the LPS-induced increase in serum NO cannot be delineated from the results of this study, it is possible that PDTC might exert its protective effect by down-regulating the expression of iNOS. Results from studies examining the role of PDTC on different models of inflammation revealed that PDTC was able to decrease the synthesis of NO by preventing the up-regulation of iNOS expression (Cuzzocrea et al., 2002; El Eter et al., 2005; Hagar et al., 2007). The molecular regulation of iNOS expression resulting in excessive amounts of serum NO released is a complex event and requires the transcription factor, NF-κB (Leist et al., 1995; Van den Berg et al., 2001). It has been shown that down-regulation of NF-κB with antioxidants resulted in decreases in the amount of NO released in the circulation, a treatment effect attributed to the reduction in iNOS expression (Hur et al., 1999). Another explanation for the reduction in serum NO level might be due to the direct scavenging effect of NO by the sulphydryl groups of PDTC. Collectively, the results of the present study demonstrated that PDTC provided protection against LPS-induced oxidative stress and acute hepatic damage via its antioxidant and...
antiinflammatory effects. PDTC may have a beneficial effect in liver injury involving oxidative stress and endotoxemia.

References


An insight into the possible protective effect of pyrrolidine dithiocarbamate